INHIBITION OF *LISTERIA* IN COLD-SMOKED SALMON USING

LIQUID SMOKE AND ISOEUGENOL

By

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ABSTRACT

Listeria monocytogenes is a foodborne pathogen, ubiquitous in nature and sometimes found in seafood. Cold-smoked salmon products have few barriers to inhibit pathogen growth. This study investigated the antilisterial effects of liquid smoke and the phenolic compound isoeugenol. Five commercial liquid smokes were tested *in vitro* and the most inhibitory to *Listeria monocytogenes* ATCC 19115 and *L. innocua* ATCC 33090 was Charsol Supreme. Chum salmon samples (100-g each) were dipped for 15 seconds at varying concentrations of liquid smoke, processed, and analyzed for *L. innocua*. Liquid smoke concentrations of 60-100% reduced *L. innocua* by 3-logs in the final product. Dwell times of 15 seconds to 5 minutes using 60% liquid smoke gradually decreased listerial survival. Isoeugenol was antilisterial *in vitro*, but lacked synergism with liquid smoke in cold-smoked salmon. Charsol Supreme formed an antilisterial barrier in cold-smoked salmon, and may be a useful application to commercial products.

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A Review of Listeria, Liquid Smoke, and Cold-smoked Salmon

Listeria's Background and Traits

The traits that give *Listeria* its own classification in *Bergey's Manual of Determinative Bacteriology* include the basic identification of a gram-positive, non-spore-forming short rod that is aerobic or facultatively anaerobic (Seeliger and Jones, 1984). Table 1 lists many other distinguishing traits of *Listeria*, including its renowned ability to grow at refrigeration temperatures. *Listeria monocytogenes* was first identified in the 1920's by two separate observations of which one group named the pathogen "*Bacterium monocytogenes*" because of the organism's ability to kill monocytes and the second group named it "*Listerella hepatolytica*" in honor of the English Lord Lister, the "father of antiseptic surgery". In 1940, the official name of *Listeria* was coined. *Listeria* had been associated with the *Corynebacteriaceae* up until 1974, and now has its own classification. The closest genus to *Listeria* is *Brochothrix* and is also closely related to *Lactobacillus*. Currently, the genus *Listeria* consists of the six species *L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri, L. seeligeri, and L. grayi* based on DNA/DNA hybridization, 16S rRNA cataloging and reverse transcriptase sequencing of 16S and 23S rRNA. Distinguishing characteristics of these species are listed in Table 2. The biochemical tests used to differentiate between *Listeria* species are hemolysis on horse blood, nitrates reduced to nitrites, the *Listeria* Christie, Atkins and Munch-Peterson (CAMP) test and production of acids from certain carbohydrates (Curtis, 1999).

Table 1. Traits of *Listeria monocytogenes*.

Optimum temperature growth	30-37°C
Temperature growth range	1-45°C
pH range	5.6-9.6
Motility	tumbling/rotatory
Catalase reaction	positive
Oxidase reaction	negative
Length	0.4-0.5μm
Diameter	0.5-2.0μm
Salt tolerance maximum	10% up to 20%
Water activity growth minimum	0.92
Water activity survival minimum	0.83
Major peptidoglycan	meso-diaminopimelic acid
Hemolysis	beta-hemolysis

Adapted from Ryser and Marth, 1999; Seeliger and Jones, 1984.

Table 2. Distinguishing Characteristics of the *Listeria* Species.

		L. monocytogenes	*L. innocua*	*L. seeligeri*	*L. welshimeri*	*L. ivanovii*	*L.grayi*
Xylose		–	–	+	+	+	–
Lactose		v	+	nd	nd	+	+
Galactose		v	–	nd	nd	v	+
Rhamnose		+	(+)	–	v	–	–
Mannitol		–	–	–	–	–	+
Hippurate Hydrolysis		+	+	nd	nd	+	–
CAMP Test	*S. aureus*	+	–	+	–	–	–
	R. equi	+	–	–	–	+	–
Beta Hemolysis		+	–	w	–	++	–
Mol % G + C		37-39	36-38	36	36	37-38	41-41
Serovars		*a*	4ab, 6a, 6b	*b*	6a, 6b	5	nd

Note: v = variable, w = weak, + = most strains positive, – = most strains negative, nd = no data

a : 1/2a, b, c; 3a, b, c; 4a, ab, b, c, d, e; "7"

b : Same as for *L monocytogenes* and *L. innocua* but no 5 or "7".

Adapted from Jay, 1996.

Listeriosis and Bacterial Invading Mechanisms

Listeria monocytogenes and possibly *L. ivanovii* have been implicated in human illness. *L. monocytogenes* infection occurs most frequently in pregnant women, neonates and people with a compromised immune system such as elderly people and cancer or AIDS patients. Listeriosis has a high fatality rate, reported between twenty and thirty percent (Fuchs and Reilly, 1992). The types of illnesses caused by a listerial infection range from diarrhea and fever in healthy adults to septicemia (in the blood stream) and meningitis (in the spinal fluid) in compromised people. The disease may cause pregnant women to abort, have a stillbirth or preterm delivery and to also have fever, diarrhea and myalgias while the neonate may have septicemia, pneumonia or meningitis. The incubation period for a *Listeria* infection is anywhere from one to twenty-one days (Ryser, 1999a). The infective dose for *L. monocytogenes* is unknown, and most likely varies from person to person. For a healthy person, it may take up to 10^8 organisms per gram to create an illness (Demetrakakes, 1999). The treatment of listeriosis is generally ampicillin or penicillin for up to six weeks.

Once inside the body, usually by ingestion, the *Listeria* bacterium attaches to the intestinal mucosa. The bacterium is either engulfed by macrophages or adheres and invades a nonphagocytic cell. The *Listeria* bacterium surface protein, internalin, is used to invade the nonphagocytic eukaryotic cell. Listeriolysin O (LLO) is used to escape the vacuole formed around the bacteria by the cell. The *Listeria* bacterium then replicates inside the cell, and prepares to move into the next cell. ActA is a bacterial surface protein that assembles the actin filaments in the eukaryotic cell to form an actin tail on one end of the bacterium that moves it around the cell at a propulsive rate up to 1.5 μm/s. With this propulsion, the bacterium comes into contact with the cell surface, and the pseudopod form is engulfed into the neighboring cell. The bacterium escapes the double vacuole in the new cell and repeats the intracellular process

(Kuhn and Goebel, 1999). In this manner, the bacterium maintains minimal contact with the immune system.

Current Regulations and Prevention of *Listeria monocytogenes*

The current regulations concerning *L. monocytogenes* in the United States, Australia and New Zealand are a zero tolerance for ready-to-eat foods. In Canada and Europe, the regulation is less than 100 cells per gram threshold depending on the food; for foods distributed to infants or susceptible people, there are more stringent specifications (Batt, 1999). Although the United States has a zero tolerance for *L. monocytogenes*, there are no current regulations under the U.S. Department of Agriculture (USDA) that require specific testing as they require for other zero tolerance pathogens like *Salmonella* and *E. coli* O157:H7 (Demetrakakes, 1999). Processors who wish to test for the *Listeria* pathogen may take swabs of the working area including the drains, or conduct product testing which is time consuming, especially for a perishable product. The United States has both a Food and Drug Administration (FDA) and a U.S. Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) method for isolating *L. monocytogenes* from food. The FDA method is geared toward *Listeria* isolation in milk and dairy products and takes 5-6 days for results whereas the USDA method targets meat and poultry while taking around 3 days for results (Donnelly, 1999; Dever et al., 1993).

The prevention of *L. monocytogenes* exposure is difficult because of its ubiquitous presence, but some basic guidelines may be followed for the consumer. These guidelines include thoroughly cooking raw food from animal sources, washing vegetables, avoiding unpasteurized milk, sanitizing hands and the work area after working with raw foods, and keeping raw meat separate from other foods. Soft cheeses should be avoided and people with a high risk of listeriosis should cook ready-to-eat foods before ingestion.

For the processor, maintaining a "clean-room" standard should eliminate problems in almost all matters (Kuhn, 1999). This standard includes eliminating cross-contamination between ready-to-eat foods and raw meat, using sanitizing dips for the employees, sanitizing the equipment, and maintaining a positive air flow in the processing plant. A hazard analysis critical control point (HACCP) plan that incorporates these procedures in the plant as well as other safety parameters will contribute to the prevention of *Listeria* contamination.

Listerial Media and Isolation and Enumeration Techniques

Listeria can be found in soil, water, vegetation, sewage or the intestinal tract. This organism is widespread, but often the numbers found are low, and isolation and enumeration techniques are required (Fenlon, 1999). The original method to isolate and enrich *Listeria* was to hold the sample at 4°C for weeks or months, ceasing the growth of many bacteria at this low temperature, while *Listeria* slowly thrived. This process was time consuming, especially in diagnosing clinical cases. There are many selective agents involved in media today that enrich and select for *Listeria*, leading to results within a couple of days. Many of the media involve ingredients such as lithium chloride, phenyl ethanol and nalidixic acid, which inhibit gram-negative bacteria, and trypaflavine and acriflavine which inhibit gram-positive cocci without detrimentally affecting the growth of *Listeria*. Some examples of enrichment media are *Listeria* Enrichment Broth (LEB), University of Vermont medium (UVM) and Fraser broth. A few selective media currently used are McBride *Listeria* Agar, Lithium chloride-Phenylethanol-Moxalactam Agar (LPM), Oxford Agar and Modified Oxford Agar. These plating media may also contain esculin, a differentiating agent that appears as a black halo of hydrolysis around each *Listeria* colony.

Many rapid detection methods have been developed and consist of immunoassays, colorimetric DNA hybridizations or immunomagnetic separations. The immunoassay and DNA hybridization both require an enrichment step whereas immunomagnetic beads can be placed into a large volume, become bound to *Listeria*, and then be resuspended into a smaller volume within 24 hours. The immunomagnetic assay is a great advantage for food processors that need to make sure their product is *Listeria*-free and still deliver the product in a reasonable time (Kohn, 1999).

Food-Borne Pathogenicity

Listeria has over the last fifteen to twenty years become a prevalent concern in food safety. Listeriosis has been known to affect people since the 1920's, but a link to food was not established until 1952, when a German researcher, H.P.R. Seeliger, linked a high number of stillbirths to the consumption of raw milk infected with *L. monocytogenes* (Seeliger, 1961). Since then, listeriosis has been reported in pasteurized milk, cheese, pate, poultry products, packaged cold cuts, vegetables, eggs and seafood products—virtually any perishable item found in a store has a potential to contain *Listeria*.

The most famous and prevalent cases in the United States involved Mexican-style cheese in the Los Angeles County in 1985, and the recent outbreak at Sara Lee's Bil Mar Foods in Zeeland, Michigan starting in December of 1998 (Kuhn, 1999). The Mexican-style cheese outbreak in Southern California affected 300 people including 85 deaths (Ryser, 1999a). The majority of the patients were pregnant women. The Center for Disease Control (CDC) was able to link a victim, a package of Mexican-style cheese found in the victim's refrigerator and a package of Mexican-style cheese bought at the victim's local market to *L. monocytogenes* contamination, which was later confirmed with genetic testing. Packaged meats and hot dogs produced by Sara Lee in 1998 were recalled nationwide, after a *Listeria* outbreak involving these

foods killed twelve people and caused three miscarriages. This incident, while creating a public scare, awakened the ready-to-eat meat industry to change or tighten protocols to prevent another outbreak, while looking for new technological solutions.

Seafood outbreaks of listeriosis are limited in number, but the potential is great. There have been many studies showing contamination of seafood with *Listeria* species including *L. monocytogenes* (Eklund et al., 1995; Dillon et al., 1994; Fuchs and Nicolaides, 1994; Jemmi, 1993; Hartemink and Georgsson, 1991). The first confirmed case of listeriosis caused by seafood was a small outbreak in 1992 in New Zealand involving smoked mussels (Brett et al., 1998). Cultures of *L. monocytogenes* from patients and an unopened packet of mussels in a patient's refrigerator were found to be identical following serogrouping and DNA macrorestriction analysis. The study showed a link between the processing factory and the outbreak finding four isolates in the factory environment of the indicated subtype. This case confirms the possibility of an outbreak in seafood.

Food Preservation

The three main categories to preserve food by inhibiting bacteria are divided into the physical, chemical and biological factors (Lou and Yousef, 1999). The physical measures include high or low temperatures and irradiation while chemical treatments may use acids, salts, liquid smoke and spices or herbs. Biopreservation methods use certain bacteria that compete and may produce bacteriocins such as nisin. *Listeria* has a high tolerance for both high and low temperatures, and survives freezing reasonably well, leaving the physical measures inadequate in many circumstances. A combination of chemical treatments may work, but a desirable food must be kept in mind when applying the chemicals, and moderation is often preferred. *Lactobacillus*

or *Carnobacterium* have been shown to successfully compete with *Listeria*, some strains producing effective bacteriocins (Nilsson et al., 1999).

Components of Smoke

Wood smoke contains over 400 different compounds (Pszczola, 1995), including carbonyls, phenolic and acidic compounds, hydrocarbons and terpenes. The carbonyls such as formaldehyde interact with proteins resulting in the color seen on smoked foods. Acidic compounds such as acetic acid are antibacterial and help lower the pH of the food product surface. The phenolic compounds may have antibacterial and antioxidant effects while also imparting color and flavors. The phenolic compounds guaiacol, eugenol, syringol, phenol, isoeugenol, cresol and xylenols compose some of these flavors (Doe et al., 1998). The polycyclic aromatic hydrocarbons (PAHs) involved in smoke contribute to the surface pellicle formation but are also known to contain the most harmful compounds, namely the carcinogen benzopyrene. Ogbadu (1999) explains the overall interaction of the smoke components:

> The formaldehyde and phenols convert the brine-solubilized protein on the food surface into a coagulated, smooth, resinous pellicle on which other smoke constituents such as tars, aldehydes, alcohols, ketones, acidic compounds and phenols are deposited.

Liquid Smoke

Liquid smoke was first produced in the 1880's, but was not manufactured until the 1970's because of previous difficulty in manufacturing the product. The current method takes the smoke produced by the combustion process of wood and mixes the smoke with cold water, to create liquid smoke. Liquid smoke contains less carcinogens than regular wood smoke; the

insoluble materials that contain the harmful PAHs are allowed to settle for 10 days and multiple filtering processes are used to produce an almost PAH-free product (Pszczola, 1995). The three forms of liquid smoke available are aqueous, vegetable-oil-based, and dry powdered. These forms may be applied to the food product by atomization, drenching, brine addition or direct addition. Liquid smoke is used more commercially than wood smoke and has a generally recognized as safe (GRAS) status from the FDA. While imparting the same flavors as wood smoke, liquid smoke is healthier for the consumer and the environment.

Cold-smoked Salmon

Cold-smoked salmon is a product continually under surveillance for potentially harboring *L. monocytogenes*. Besides a thermal process that never exceeds 28°C, salmon is also a ready-to-eat food that is not reheated before consumption. The barriers against possible pathogenic bacterial growth are usually not enough to prevent proliferation. The act of smoking does produce a partial barrier due to the combination of drying and the chemical components of the smoke (Daun, 1979). The processing of cold-smoked salmon as deemed by the Association of Food and Drug Officials (AFDO, 1991) states the temperature in the smoking chamber does not exceed 28°C for a drying and smoking period not exceeding 20 hours. Air packaged fish must contain at least 2.5% water-phase salt and for vacuum or modified atmosphere packaging, the fish must contain at least 3.5% water-phase salt to prevent *Clostridium botulinum* growth and toxin formation in the product. Modified atmosphere packaging (MAP) seems to increase the growth rate of *L. monocytogenes* (Lou and Yousef, 1999) and a study of vacuum-packed smoked salmon found *L. monocytogenes* to grow well in storage conditions (Rorvik et al., 1991). These findings show the dangers in vacuum-packaged products that rely mostly on refrigerated temperatures that are "safe" for an extended period of time.

Experimental Study

A cold-smoked salmon outbreak with *Listeria* has not occurred, but the presence of the bacteria in this food product has been well documented (Eklund et al., 1995; Fuchs and Nicolaides, 1994; Jemmi, 1993). There are guidelines but not many regulations by the FDA regarding cold-smoked salmon, leaving gaps as to the safety of each cold-smoked salmon product. The potential for an outbreak in cold-smoked salmon encouraged scientific studies in this field. Salt, nisin, bacteriocins, sodium nitrite and smoke have all been studied, some in conjunction with others, to try and prevent listerial growth. The objective of this project was to evaluate different liquid smokes and work with the most *Listeria*-inhibitory smoke in the cold-smoked salmon product, and to determine if isoeugenol could be used in conjunction with the liquid smoke. Producing a safe and palatable product for the consumer was considered and sensory evaluation scores determined the product desirability. This study takes a step in finding a cold-smoked salmon process desirable to consumers while acting as an effective listerial inhibitor.

Inhibition of *Listeria* in Cold-smoked Salmon Using Liquid Smoke and Isoeugenol

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Abstract

Five commercial liquid smokes were tested *in vitro* and the most inhibitory to *Listeria monocytogenes* ATCC 19115 and *L. innocua* ATCC 33090 was Charsol Supreme. Chum salmon samples (100-g each) were dipped for 15 seconds at varying concentrations of liquid smoke, processed, and analyzed for *L. innocua*. Liquid smoke concentrations of 60-100% reduced *L. innocua* by 3-logs in the final product. Dwell times of 15 seconds to 5 minutes using 60% liquid smoke gradually decreased listerial survival, with an optimum dwell time of 5 minutes. Panelists found the 0 to 2 minute dwell times slightly desirable with no significant differences. The 5 minute treatment was significantly darker and scored lower in desirability and flavor. Isoeugenol was antilisterial *in vitro*, but lacked synergism with liquid smoke in cold-smoked salmon. Charsol Supreme formed an antilisterial barrier in cold-smoked salmon, and may be a useful application to commercial products.

Key Words: cold-smoked salmon, *Listeria monocytogenes*, liquid smoke

Introduction

The omnipresence of *L. monocytogenes* has made this pathogen a food safety hazard in vegetable, dairy, seafood and meat products (Brackett, 1999; Ryser, 1999b; Farber, 1991; Truscott and McNab, 1988). Cold-smoked salmon is a primary concern in the seafood industry, because the process lacks a temperature high enough to kill *Listeria*, and is packaged ready-to-eat. Although *L. monocytogenes* has not caused any outbreaks in cold-smoked salmon, *L. monocytogenes*' presence has been confirmed in an average 10% of sampled products (Jemmi, 1993). Liquid smoke has been studied in smoked salmon (Niedziela et al., 1998; Thurette et al., 1998; Poysky et al., 1997) and wiener exudates and beef franks (Faith et al., 1992; Messina et al., 1988) as a possible barrier against *Listeria*. Smoking can impart desirable flavors and coloring

with the right application. Many of the carcinogenic compounds have been taken out of liquid smoke, and the use of liquid smoke commercially has displaced wood smoking. The objectives of this study were to find a liquid smoke inhibitory to *L. monocytogenes*, apply this smoke to cold-smoked salmon, test isoeugenol with salmon, and evaluate reduction in the levels of *Listeria*.

Materials and Methods

Cultures

Listeria monocytogenes ATTC 19115 and *L. innocua* ATTC 33090 were used in the experiments. These stock cultures were stored in a -70° C freezer. After the vials were partially thawed, a sterile loop of each culture was transferred to tubes containing 2 ml brain heart infusion (BHI; Difco, Detroit, MI) and incubated overnight at 35°C. A loop from each tube was streaked on BHI plates and incubated overnight at 35°C. Cultures for each of the assays were generated from colonies of current plates.

Liquid Smoke and Isoeugenol Screening

Five liquid smokes produced by Red Arrow (Manitowoc, WI) were screened for antilisterial activity. The smokes used were Charsol H-10, Aro-Smoke P-50, LFB Poly Supreme, CharOil and Charsol Supreme. Aseptic dilutions of smoke using sterile water to form concentrations of 2, 5, 10, 25, 50 and 100% were made in one ml aliquots. In triplicate, various smoke concentrations (0.1 ml : 2.0 ml BHI) were tested. *L. monocytogenes* and *L. innocua* inoculated tubes and a control set were incubated at 35°C overnight.

Isoeugenol (Aldrich, Milwaukee, WI) was diluted in absolute ethanol to 1% (v/v) and various concentrations (0 to 400 ppm) were tested in a final volume of 2 ml. The tubes were incubated at 35°C for 22 hours.

All absorbancies were read at 600 nm for growth (Spectronic 710, Bausch & Lomb, Rochester, NY).

Cold-Smoked Salmon Experiment

An inoculum was prepared from an overnight culture (50 ml BHI) of *L. innocua* which was centrifuged (9,000 x g, 10 min., 15°C), washed and resuspended to half the original volume in 0.1% (w/v) peptone water.

Frozen dark chum salmon (*Oncorhynchus keta*) fillets were thawed overnight at 4°C. The filleted fish were cut into 100-g pieces, brined (20% salt) for 35 minutes and briefly rinsed.

Liquid smoke was applied either by spraying (2 ml using a 25% concentration) or dipping in 300 ml of 40, 60, 80 and 100% concentrations. The inoculum (0.5 ml) was applied to the salmon pieces and spread with a sterile hockey stick. Salmon pieces were placed in the Enviro-Pak Model CHU-150 commercial smoker (Clackamas, OR) and processed for 16 hours at 21°C. Salmon pieces were collected from all treatments prior to and after the smokehouse and placed in bags (Whirl-pak, Nasco, Fort Atkinson, WI) for microbial and non-microbial analyses.

Dwell Time Experiment

Salmon pieces were dipped (0–5 min) in 60% Charsol Supreme and microbial analysis was performed on the post smokehouse samples as described below.

Isoeugenol, Liquid Smoke and Cold-Smoked Salmon

Salmon pieces were dipped for one minute in ethanol (control) or in 300 ml of isoeugenol (400 and 4000 ppm in ethanol). In a separate experiment, salmon pieces were dipped for five minutes in 60% Charsol Supreme, 4000 ppm isoeugenol, or a combination. All pieces were inoculated and processed. Samples were collected and microbial analysis performed as described below.

Microbial Analysis

Salmon pieces (25-g) were aseptically cut, diluted ten-fold and stomached (Stomacher Lab-Blender 400 Tekmar Co., Cincinnati, OH) for two minutes. Serial dilutions in 0.1% peptone water were made with the inoculum and the stomached samples and spread-plated in duplicate on modified oxford (mOXF; Difco, Detroit, Michigan). All plates were incubated at 35°C for 48 hours and colonies were counted.

Non-Microbial Analysis

The salmon samples were measured for color and then ground with an Osterizer® (Sunbeam, Boca Raton, FL) blender for the analysis of moisture content, salt content and pH. For color, L*a*b* values were recorded of the external portions of the salmon pieces using a Minolta Chroma Reflectance Meter II (Minolta Camera Corp., Ramsey, NJ). Means and standard deviations were calculated for ten readings per piece. For moisture content, triplicate samples were dried at 102°F overnight (AOAC method 950.46B; Imperial IV Microprocessor, Lab-Line Instruments, Inc., Melrose Park, IL), and dry weights were calculated. For salt content,

duplicate samples (10-g) were blended for 30 seconds with 90 ml of boiling water, and salt content was determined using QUANTAB® Chloride Titrator (AOAC method 976.19; ETS, Elkhart, IN). For each salmon sample, water-phase salt (WPS) was calculated (Hildebrand, 1992). For pH, duplicate 1:10 samples to distilled water were mixed and pH recorded (Model 125, Corning Glass Works, Medfield, MA).

Sensory Panel.

An experienced panel of nine evaluated five samples of cold-smoked red salmon (*Orcorhynchus nerka*). The samples were dipped in a 60% Charsol Supreme solution for 0, 5 sec, 15 sec, 2 min and five min and dried in the smokehouse as described above. Sensory scores were evaluated for odor (7 = extremely pleasant, 1 = extreme off odor), flavor (7 = extremely pleasant, 1 = extreme off flavor), color (7 = very dark, 1 = very light), salt (7 = very salty, 1 = very bland) and desirability (7 = extremely desirable, 1 = extremely undesirable). The sensory evaluation data was analyzed using analysis of variance with $p < 0.05$ (Statistica 5.1, Stat Soft Inc., Tulsa, OK). Differences between treatments were evaluated using LSD to determine significance.

Results and Discussion

Of the five liquid smokes tested for antilisterial activity in test tubes, Charsol Supreme gave the highest inhibitory results (Table 3) and was chosen for use in the cold-smoking salmon experiments. The specifications of the liquid smokes (Table 4) indicate that Charsol Supreme has a higher acidity and more smoke flavor compounds and carbonyls combined than the other smokes. Acidity (acetic acid), smoke flavor compounds (phenols), and carbonyls (formaldehyde) have all been linked to inhibition of bacteria (Faith et al., 1992; Daun, 1979).

Since *L. innocua* can survive similar conditions to that of *L. monocytogenes* as indicated by the tube assays (data not shown), and studies performed (Eklund et al., 1995; Weagant et al., 1988), *L. innocua* was used in the cold-smoking process for safety. At first, a spray design of Charsol Supreme at 25% was used to coat the salmon, but was discontinued because of difficulty with the sprayer clogging and the results were insignificant with a one log decrease of *L. innocua*. Whereas a liquid smoke concentration of 0.5% inhibited *L. innocua in vitro*, a much higher concentration of liquid smoke was required for the cold-smoked salmon experiment and may be due to the complex proteins in the salmon that react with the acetic acid and other components of the liquid smoke. Dipping in a 100% solution of Charsol Supreme for 15 seconds gave a 3-log bacterial reduction from the pre-smokehouse non-smoked salmon to the post-smokehouse smoked salmon. Decreasing the concentration to 60% gave similar results to the 100% (Figure 1), leading to the use of the less concentrated solution in further experiments. In raw milk (Fenlon, 1999), fresh picked blue crabmeat (Rawles et al., 1995), and shrimp and lobster meat (Farber, 1991), *L. monocytogenes* occurred at <35 CFU/ml, <100 CFU/ g, and <10 MPN/g respectively. If salmon naturally contained <100 CFU/g of *L. monocytogenes*, the liquid smoke and smokehouse process would in theory eliminate all of the pathogenic bacteria. The liquid smoke may also decrease post-processing contamination.

The values for color are shown in Table 5. The L* value measures from white to black, a higher number indicating a whiter color. The a* value measures from green (-) to red (+) and the b* value measures from yellow (+) to blue (-). The L*a*b* values do not widely differ between concentrations. The pre-smokehouse non-smoked samples were the lightest in color, while the pre-smokehouse smoked samples and post-smokehouse non-smoked samples were around the same color intensity, and the post-smokehouse smoked samples were the darkest. The liquid smoke seemed to even out any differences in the a* values from piece to piece variation.

The b* value was similar to all pieces except the pre-smokehouse smoke-dipped sample had a high value of around 21, which indicated more yellow coloring than the other pieces.

The values for pH, moisture and water-phase salt are shown in Table 6. The pH of the smoke-dipped salmon is 5.27 in 100% smoke and 5.61 in 60% smoke. These values were of the total ground samples, indicating the pH of the salmon surface would likely be lower than these values and below the growth pH limit for *L. monocytogenes*, contributing to the decrease in *Listeria*. The salt content stayed below 3.0 % for most of the samples. Consumers prefer a salt value of around 2.0-3.0%, but a certain amount of salt is required to keep the WPS elevated. The smoke-dipped samples contained less salt than the controls in all but one sample and may have displaced some of the salt in the salmon pieces. The moisture content of the brined salmon pieces initially was 80%. After dipping the brined salmon pieces in liquid smoke, the moisture content dropped to around 76%. Drying decreased the moisture to around 61% in the non-smoked salmon pieces and 63% in the smoked pieces. The WPS calculations range from 2% to 5%. Cold-smoked salmon is usually a vacuum packaged product, and the salmon should contain at least a 3.5% WPS to prevent *C. botulinum* growth and toxin production (AFDO, 1991). This product would have to be continually monitored to assure a satisfactory WPS percentage.

Dwell time trials of the post-smokehouse salmon showed a gradual decrease in microbial survival (Figure 2). The microbial numbers varied toward a gradual decrease with variation possibly due to the salmon pieces. A possible contributor to variation would be higher moisture content in one piece compared to another that allowed for better microbial growth. Although the manufacturer recommended atomization of Charsol Supreme, a 60% dip was used. The 5 minute dipping of salmon was very dark compared to the non-smoked and the 15 second dipped salmon.

The *in vitro* assay showed inhibition of *L. innocua* at 200 ppm and 400 ppm isoeugenol (Figure 3), similar to and confirming the results of Faith et al. (1992) that isoeugenol is

antilisterial. Applied to salmon, isoeugenol showed a one-log reduction of *L. innocua* at the 4000 ppm (Table 7). This amount of isoeugenol was combined with liquid smoke as a possible additive barrier to bacterial survival on salmon. The experiment combining 60% Charsol Supreme and 4000 ppm isoeugenol showed unexpected results. The 60% smoke inhibited *L. innocua* more than the smoke and isoeugenol combination (Table 8). The smoke and isoeugenol mixture created a dark coating on the salmon, the sides of the beaker and onto the forceps used for dipping. The interaction of the isoeugenol and liquid smoke created a less effective dip than the original smoke dip, and the tar-like substance may have contributed to the loss of antibacterial activity. The isoeugenol may have precipitated out some of the antimicrobial components making them useless.

The sensory evaluation revealed that cold-smoked salmon up to a two minute liquid smoke dip was desirable, but the five minute dip was less than desirable (Table 9). The panelists preferred the odor of the five second dip, and gave the lowest score to the five minute dip. The color was the lightest in the non-smoked and darkest in the five minute dipped salmon, with panelists preferring the "pleasing" red color on the non-smoked and five second dipped samples. The salt evaluation was similar in all of the results and panelists commented that the salmon was either slightly too salty or not quite salty enough. In both flavor and desirability, panelists preferred the non-smoked salmon and the two minute dipped salmon. Flavor was the lowest for the five minute dipped salmon with panelists' comments of bitterness and strong aftertaste. The longer dip time would be favorable for the reduction of *Listeria*, but the panelists revealed that the five minute dip was not a product they would desire. The two minute dip time was as desirable as the other samples and would be considered the best product.

Conclusion

Of five liquid smokes tested in this study, Charsol Supreme effectively reduced the number of *Listeria* in processed cold-smoked salmon from 10^6 to 10^3 CFU/g. Further studies using a smaller initial and more realistic inoculation load (10^3) may prove to eliminate the *Listeria* bacterium altogether. Isoeugenol did not work synergistically with the liquid smoke, but an application of isoeugenol in a different solution may enhance the killing ability. The sensory evaluation revealed that cold-smoked salmon dipped for two minutes in 60% Charsol Supreme was as desirable as salmon dipped for a reduced period of time, but the five minute dip was undesirable. This study found that using liquid smoke on cold-smoked salmon can provide an edible product while ensuring a safer food. Further study in trying to prevent listerial growth in cold-smoked salmon is needed, focusing on a safe food product and considering the consumer reaction.

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Table 3. Inhibition of *Listeria monocytogenes* and *L. innocua in vitro* by commercial liquid smokes.

% Smoke	Charsol Supreme	Charsol H-10	Charsol LFB Poly	Aro-Smoke P-50	Charoil
0%	+	+	+	+	+
0.1%	+	+	+	+	+
0.25%	+	+	+	+	+
0.50%	–	+	+	+	+
1.25%	–	–	–	+	+
2.50%	–	–	–	–	+
5.00%	–	–	–	–	–

+ = growth, – = no growth

Table 4. Comparison of components for five commercial liquid smokes.

	Charsol Supreme	Charsol H-10	Charsol LFB Poly	Aro-Smoke P-50	Charoil
pH	2.1-2.6	2.1-2.6	2.0-2.4	3.0-4.0	7.3-8.1
Total acidity (% acetic acid)	14.0-16.0	10.5-12.0	13.0-15.0	4.0 max.	n.d.
Smoke Flavor Compounds (mg/ml)	18.0-25.0	10.0-15.0	9.0-14.0	37.0-42.0	n.d.
Carbonyls (%)	20.0-25.0	12.0-13.0	16.0-20.0	n.d.	n.d.
Density (lb/gal)	9.3	8.9	9.3	9.0	7.6

n.d.= no data

Adapted from Red Arrow Specification Sheets (1998).

Table 5. Color[1] of pre- and post-cold-smoked salmon dipped in different concentrations of Charsol Supreme.

		100%	80%	60%
Pre N.S.	L*	55.9 ± 5.04	53.3 ± 3.42	58.8 ± 4.20
	a*	3.2 ± 2.81	5.0 ± 1.19	0.6 ± 1.46
	b*	8.8 ± 3.52	10.5 ± 1.57	9.6 ± 4.59
Pre SMK	L*	51.2 ± 2.45	48.6 ± 2.07	49.4 ± 2.66
	a*	5.0 ± 1.95	6.8 ± 1.39	6.7 ± 1.40
	b*	20.8 ± 3.18	21.0 ± 2.30	21.2 ± 2.12
Post N.S.	L*	49.5 ± 2.20	50.1 ± 2.51	55.4 ± 3.71
	a*	6.0 ± 1.70	8.0 ± 1.03	3.7 ± 0.91
	b*	9.1 ± 2.18	10.5 ± 1.54	12.9 ± 4.67
Post SMK	L*	43.3 ± 1.67	44.9 ± 1.81	44.4 ± 2.00
	a*	5.8 ± 0.87	6.4 ± 1.29	5.6 ± 1.26
	b*	8.0 ± 2.66	7.8 ± 2.08	7.6 ± 3.47

Pre N.S. = pre-smoker, no liquid smoke

Post N.S. = post-smoker, no liquid smoke

Pre SMK = pre-smoker, with liquid smoke

Post SMK = post-smoker, with liquid smoke

[1] n=10

Table 6. Proximate analysis of pre- and post-smoked salmon dipped in different concentrations of Charsol Supreme.

	pH[1]			% Moisture[2]			% Salt[1]			% Water-Phase Salt		
Smoke Concentrations	**100%**	**80%**	**60%**	**100%**	**80%**	**60%**	**100%**	**80%**	**60%**	**100%**	**80%**	**60%**
Pre N.S.	6.35	n.d.	6.43	79	79	80	1.84	1.48	1.98	2.28	1.84	2.43
Pre SMK	5.24	n.d.	5.73	77	75	76	1.71	1.20	2.48	2.17	1.59	3.10
Post N.S.	6.27	n.d.	6.34	61	59	62	3.38	2.38	3.17	5.25	3.88	4.88
Post SMK	5.27	n.d.	5.61	63	61	64	2.77	1.98	2.92	4.21	3.14	4.39

See footnote 5 for definitions. n.d. = not determined

[1] n=2

[2] n=3

Table 7. Effects of isoeugenol on *L. innocua* (CFU/g) in salmon without liquid smoke.

Pre-ethanol	3.24×10^5	Post-ethanol	1.43×10^5
Pre-400 ppm	5.75×10^5	Post-400 ppm	7.0×10^4
Pre-4000 ppm	5.5×10^5	Post-4000 ppm	2.4×10^4

Pre- = pre-smoker, Post- = post-smoker

Table 8. Effects of isoeugenol and liquid smoke on *L. innocua* (CFU/g) in cold-smoked salmon.

	Trial 1	Trial 2
Control (no isoeugenol or smoke)	3.5×10^4	7.35×10^5
4000 ppm isoeugenol	9.6×10^3	1.79×10^4
60% smoke	≤ 100	≤ 10
60% smoke + 4000 ppm isoeugenol	1.28×10^3	2.3×10^2

Table 9. Sensory Evaluation[1] of Cold-smoked Salmon Dipped in 60% Liquid Smoke.

	Odor	Flavor	Color	Salt	Desirability
Non-smoked	5.1[a]	5.6[a]	3.6[a]	4.9[a]	5.2[a]
5 second	6.2[a]	4.6[a]	5.2[b]	5.3[a]	5.0[a]
15 second	5.7[a]	4.6[a]	5.0[b]	5.3[a]	4.4[a]
2 minute	5.6[a]	5.3[a]	5.7[bc]	5.4[a]	5.3[a]
5 minute	4.9[a]	3.0[b]	6.6[c]	5.3[a]	2.7[b]

[1] n=9

Values in a column with the same superscript were not significantly ($p<.05$) different from one another.

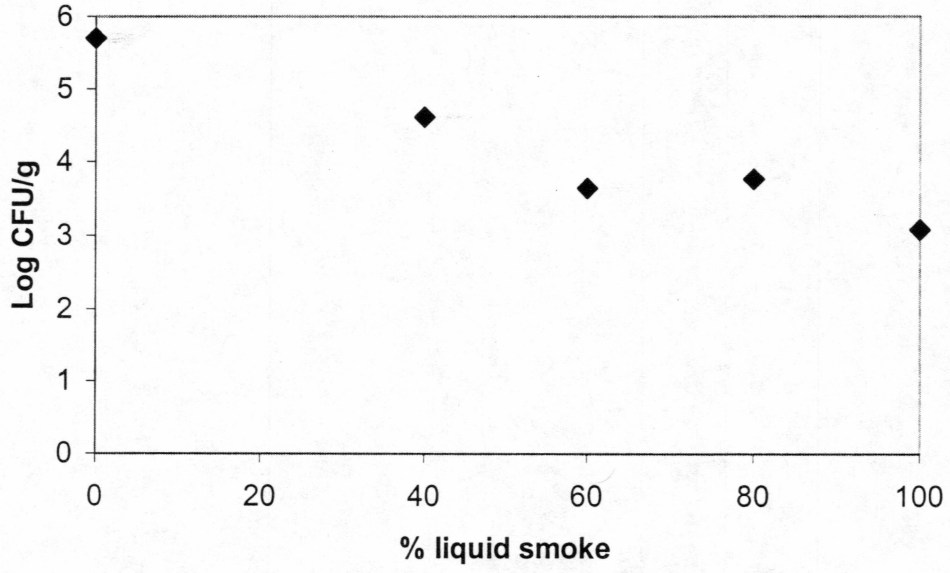

Figure 1. Effect of Charsol Supreme concentrations on *L. innocua* survival in cold-smoked salmon.

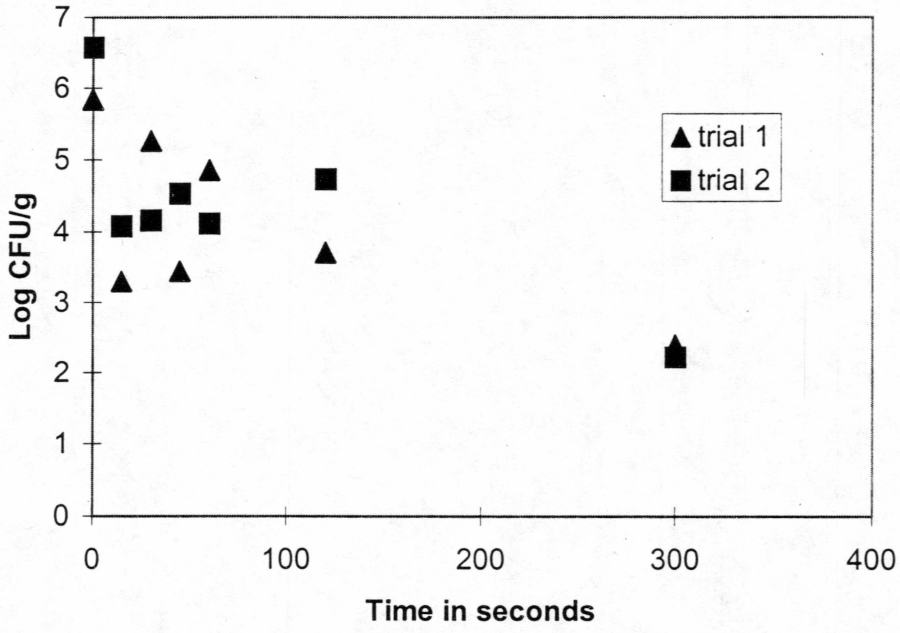

Figure 2. Effect of 60% liquid smoke at different dwell times on *L. innocua* survival.

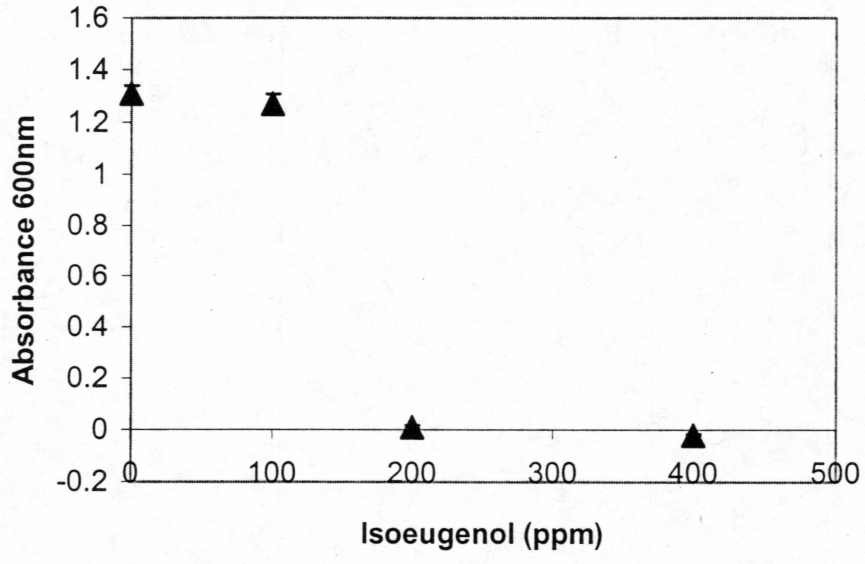

Figure 3. Effect of isoeugenol concentrations on *L. innocua* survival in brain heart infusion broth.